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Type studies of *Russula* species described by W.A. Murrill, 1. *R. roseiisabellina*, *R. sericella,* and *R. obscuriformis*

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Abstract — The study of the North American russulas remains severely handicapped by the lack of sufficient microscopic detail on approximately one third of the described taxa. In this contribution, microscopic features are examined and illustrated in detail for three of Murrill's type specimens. The authors reinterpret these data in combination with the macroscopic features supplied in the original diagnoses and propose a more appropriate infrageneric placement of the taxa: (i) *R. roseiisabellina* is not close to *R. sericeonitens* (subgenus *Russula*) as suggested in Murrill's protologue, nor is it a synonym of *R. rimosa* (subgenus *Heterophyllidia*) as suggested by Hesler — it is a typical member of the *R. foetens* group (subgenus *Ingratula*); (ii) *R. sericella* is absolutely unrelated to *R. obscuriformis* as suggested in Murrill's protologue and probably fits within subgenus *Russula* or *Tenellula*; and (iii) *R. obscuriformis* is similar to the recently described *R. texensis* in *Russula* sect. *Xerampelinae*, but new collections are needed to verify whether it possesses the typical field characters of this group.

Key words — Florida, micromorphology, *Russulaceae*, taxonomy

Introduction

William Murrill (1869–1957) was without any doubt one of the most colorful and influential personalities among 20th century mycologists in America (Weber 1961). His prolific description of new taxa has given him a very controversial reputation, also in the case of *Russula* Pers., and Murrill surpasses by far his fellow mycologists with a total of 111 newly described *Russula* species, most collected near Gainesville, Florida (Buyck 2007).

As a professional mycologist, Murrill started his career at the New York Botanical Garden, where he was probably influenced and stimulated towards description of new taxa by the nomenclatural views and ambitions of the newly imposed "American Code for nomenclature" at the New York Botanical

Garden (Weber 1961). Murrill usually published his new russulas without any critical revision or discussion of related or similar taxa and without placing or grouping them into a subgeneric classification scheme. His descriptions were very short and mainly oriented towards field characters at a time when the study of the microscopic features of European *Russula* (Maire 1910, Melzer 1934) had already reached the degree of precision that is still in use today. Judging from published synonymies (Bills 1984, Bills & Miller 1984) and our own experience with his collections, it is therefore not surprising that Murrill had probably difficulties in recognizing his own taxa (see commentary under *R. roseiisabellina* below for example).

Hesler (1960, 1961) and Singer (1947, 1958) were the only mycologists who ever tried to add something more substantial to Murrill's original diagnoses. Hesler published very short notes on microscopic features for many of Murrill's types, but even the kind of precision Hesler added for spores and elements of the pileipellis is still largely insufficient to allow for good interpretations and comparisons with modern descriptions of *Russula*. More recently, a few other mycologists (e.g., Bills 1984, Bills & Miller 1984, Kibby & Fatto 1990) have studied at least some of Murrill's collections but mostly without publishing the features they examined on Murrill's types. In a few instances, these re-examinations eventually resulted in proposed synonymies or different interpretations for some of Murrill's taxa.

With taxonomic expertise on *Russula* having literally vanished in North America over the past decennia (Buyck 2007) and in the absence of precise data on Murrill's types, the study of the North American russulas remains severely handicapped by the lack of sufficient microscopic detail on approximately one third of the described russulas on the continent.

This paper is the second of a series of detailed and illustrated microscopic revisions of Murrill's taxa in an effort to promote and stimulate rapid progress in the study of American *Russula* (see Adamčík & Buyck 2010). We also try to place the examined species in the latest available (mainly European) classifications of the genus. And even if such a placement remains very tentative in the absence of more precise data on fresh material, our observations suggest in most cases very different affinities for most of the examined species.

One character shared by nearly all taxa in this contribution concerns the color change on bruising or on drying: nearly all examined taxa supposedly exhibit a distinct browning of at least part of the fruit bodies: *R. roseiisabellina* (Murrill 1943) has lamellae "becoming brownish where bruised", *R. sericella* (Murrill 1945) was described with a stipe "becoming pale-brownish when handled". Finally, we also include here *R. obscuriformis* (Murrill 1945), which was considered to be a synonym of *R. sericella* by Singer (Singer in sched.).

Materials & methods

Micromorphological characters were observed in Olympus CX-41 and Nikon Eclipse E400 microscopes using an oil-immersion lens at a magnification of 1000×. All drawings of microscopical structures – except of spores – were made with 'camera lucida' using a Nikon Y-IDT drawing attachment at a projection scale of 2400×. Contents of hymenial cystidia and pileocystidia in the illustrations are indicated schematically, except for a single element where contents are indicated as observed in Congo red preparations from dried material. Spores on the lamellae were observed in Melzer's reagent. All other microscopic observations were made in ammoniacal Congo red, after a short aqueous KOH pre-treatment to improve tissue dissociation through gelatinous matrix dissolution. All tissues were also examined for the presence of ortho- or metachromatic contents or incrustations in cresyl blue as explained in Buyck (1989).

Spores were scanned with an Olympus Artcam camera and measured using Quick Micro Photo (version 2.1) software. Enlarged scanned pictures of spores were used for measuring with an accuracy of 0.1 µm and for drawing. Q gives length/width ratio of the spores. Measurements exclude ornamentation. Statistics for measurements of microscopical characters are given as mean value (boldface) plus/minus standard deviation and are based on 30 measurements. Values in parentheses give measured minimum or maximum values. An estimate for spore ornamentation density is given following Adamčík & Marhold (2000).

Names for infrageneric taxa follow the classification system proposed by Romagnesi (1985, 1987).

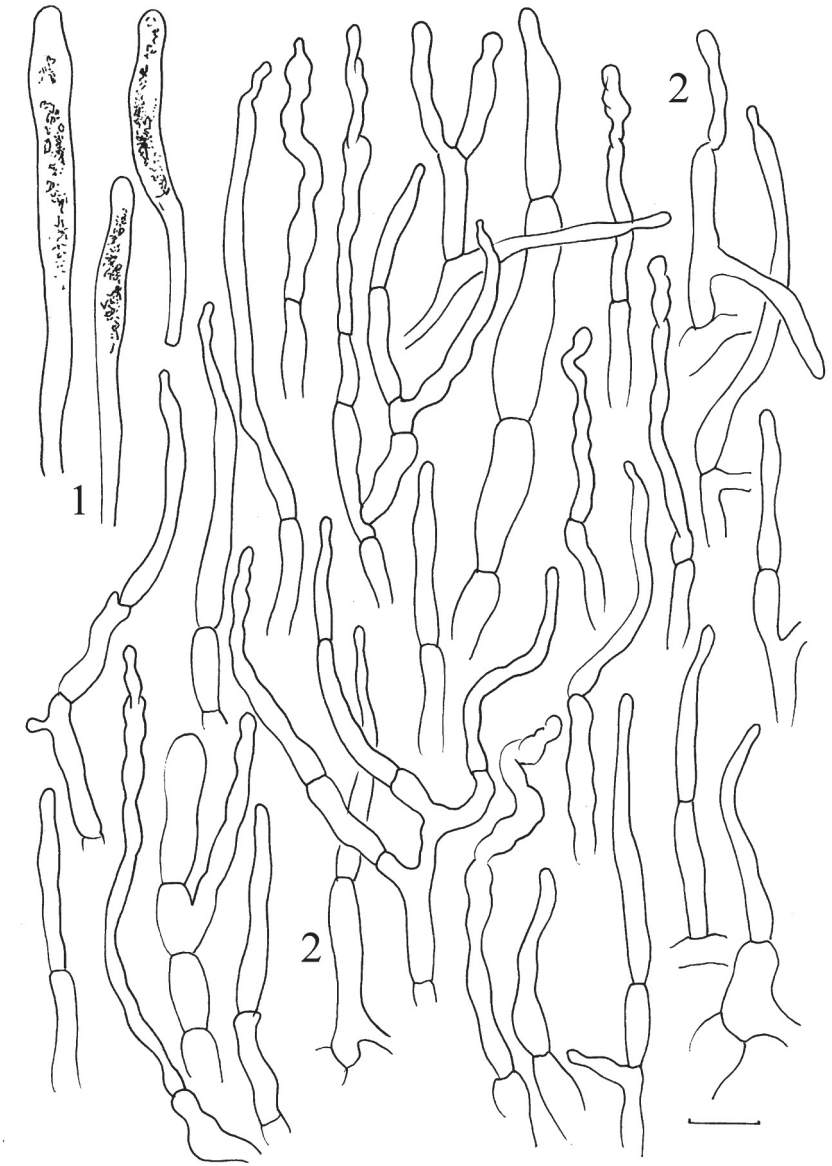
Taxonomy

Russula obscuriformis Murrill, Lloydia 7(4): 312. 1945. Figs 1–6

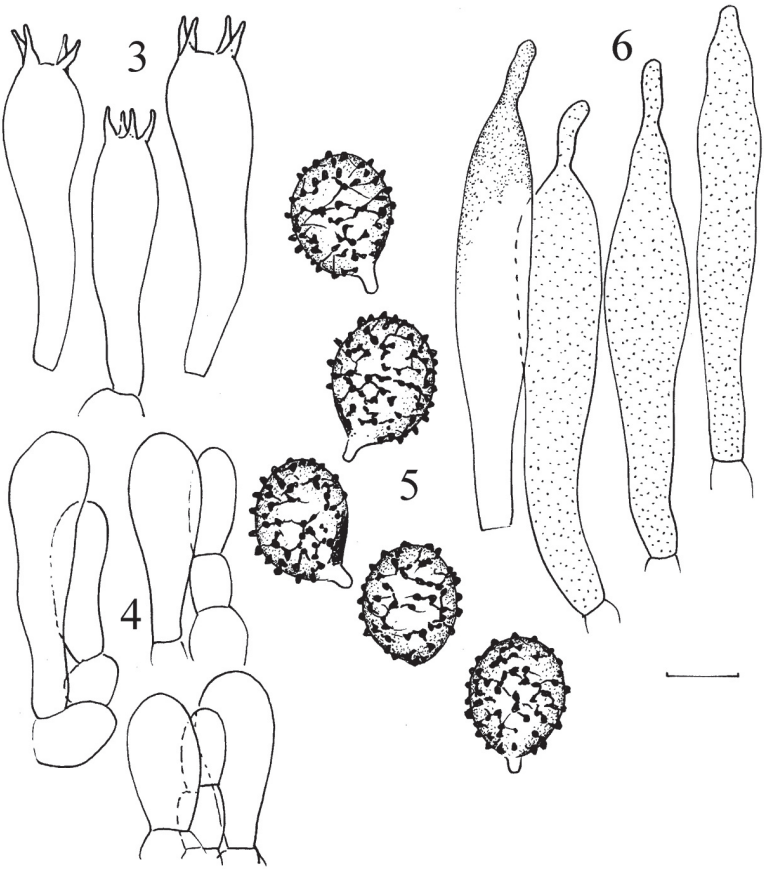
Original description and typification — Pileus convex to slightly depressed, solitary, 8 cm broad; surface dry, smooth, at length glabrous, dark-roseous with bay center, darker on drying, margin even or slightly striate, entire, peeling readily; context thin, white, discolored on drying, odorless, mild; lamellae adnate, mostly equal, few forked, broad, medium distant, entire, white; spores subglobose to broadly ellipsoid, finely tuberculate, stramineous in mass, about 8 µm long; cystidia none; stipe equal, smooth, glabrous, white, 5 × 2 cm.

Type collected by W. A. Murrill under an oak at LaCrosse, Alachua Co., Fla., July 13, 1938 (F 15536). Suggesting *R. obscura* Romell but differing in color and spore color. The gills in dried specimens are distinctly gray. [Holotype at FLAS sub nr. F15536].

Microscopic features — Spores broadly ellipsoid, (7.3–)7.7–**8.1**–8.4(–8.7) × (6.2–)6.4–**6.7**–7(–7.4) µm, Q = (1.11–)1.15–**1.2**–1.25(–1.27); ornamentation rather dense, subreticulate to reticulate, composed of conical to hemispherical, often large, strongly amyloid warts, ca (3–)5–7(–8) warts in a 3 µm diam. circle, measuring 0.5–0.7 µm high, interconnected by fine line connections (2–6 in a circle) or fused in ridges (0–4 fusions in the circle); suprahilar plage amyloid. basidia (32–)37.5–**40.5**–43.5(–45) × (10–)11–**12**–13(–13.5) µm, 4-spored, clavate. Subhymenium pseudoparenchymatic. Lamellar trama mainly composed of large spherocytes. Hymenial cystidia widely dispersed, less



Figs. 1–2. *Russula obscuriformis* (holotype). 1. Pileocystidia, with contents indicated in one element as seen in Congo red. 2. Hyphal terminations near cap margin. Scale bar = 10 µm.



Figs. 3–6. *Russula obscuriformis* (holotype). 3. Basidia. 4. Basidiola. 5. Spores in Melzer's reagent. 6. Hymenial cystidia with contents indicated in one element as seen in Congo red.
Scale bar = 5 μm for spores, 10 μm for the other elements.

than 500/mm^2 and very difficult to observe except near gill edge, measuring ca. 56–80 × 9–14 μm on sides, clavate to fusiform-pedicellate, mucronate-appendiculate, thin-walled, with few and ill-defined, SV-negative contents that are not strongly refringent in KOH. Marginal cells not differentiated. Pileipellis orthochromatic in cresyl blue, without incrustations, not sharply delimited from the underlying spherocytes of the context, vaguely divided in a rather poorly gelatinized subpellis and a more dense suprapellis of intricate to ascendant hyphae, with poorly differentiated pileocystidia. Hyphal endings thin-walled and easily collapsing, near margin with terminal cells measuring (19–)24.5–**34.2**–44(–55) × (2.5–)3–**3.2**–4(–4.5) μm, slender, attenuated or

cylindrical, with apex often more or less abruptly, irregularly or repeatedly constricted and only 1.5–2.5 µm diam., occasionally clavate; subapical cells shorter and often somewhat larger in diam., often branched. Pileocystidia scarce and indistinct, single-celled, arising from the subpellis, cylindrical to subclavate, ca. 4–5.5(7.5) µm wide, with poorly differentiated, granular contents that do not react to sulphovanillin. Clamp connections absent in all parts.

Commentary — *R. obscuriformis* differs from all other species discussed in this paper by the spores that are "stramineous in mass" – an observation that confirms at least that Murrill did distinguish between pale and darker spore prints in *Russula*. It is therefore difficult to understand why Singer synonymizes this species with *R. sericella* (having a white spore print), which has indeed a completely different pileipellis under the microscope.

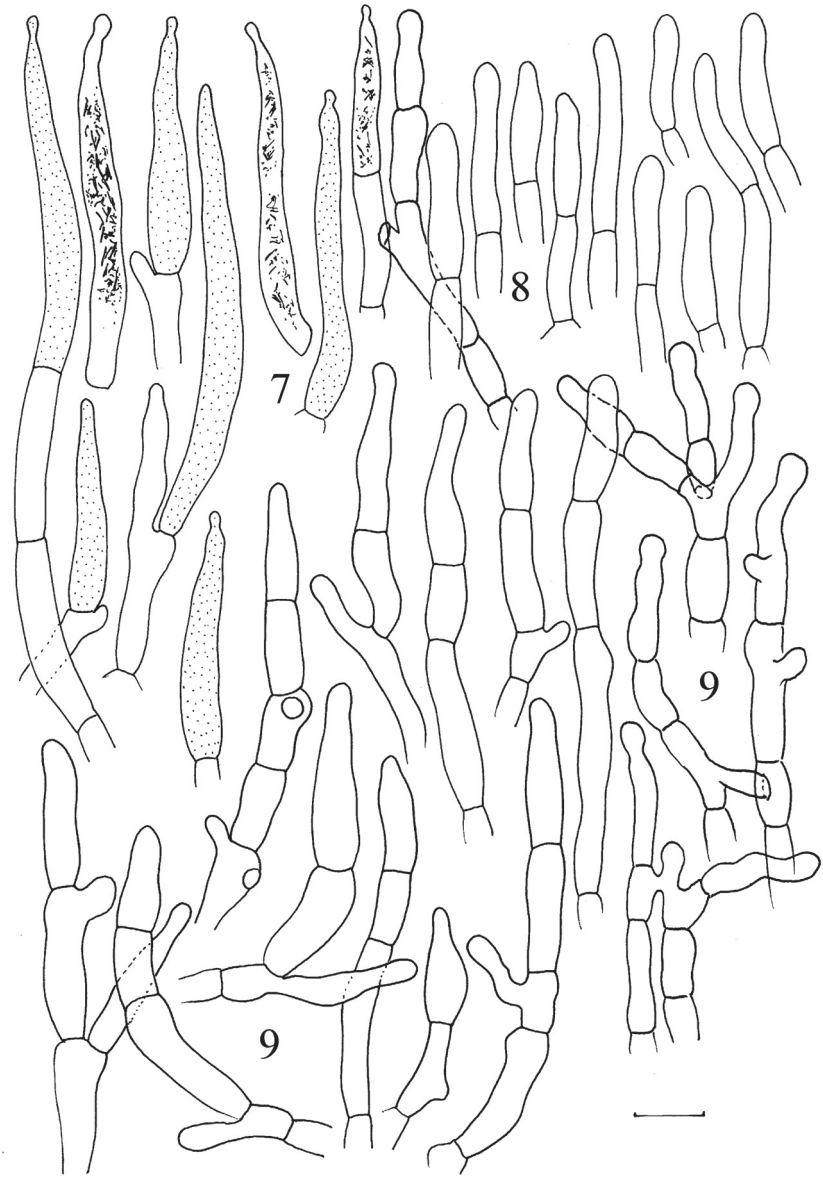
The discoloring context should, in our opinion, be interpreted as 'graying' context because of the comparison to "*R. obscura* Romell" (= *R. vinosa* Lindblad) and the mention of the distinctly grayish lamellae on drying. This graying context, together with the odorless flesh and the very prominent reticulation of the spores, is a combination of characters that does not suggest a place in *Russula* sect. *Xerampelinae* (Singer) Jul. Schäff. However, the recently described *R. texensis* (Buyck et al. 2008) is a fishy *Russula* with grayish-brownish discoloring context and a weak fishy smell. It has an overall similar color and also similar features of spores and pileipellis. The two taxa differ nevertheless by the much more pronounced reticulation of the spores in *R. obscuriformis* and the very scarce, smaller and hardly prominent pileocystidia. The spore print color of *R. texensis* was noted as 'pale' on the gills (a sufficient spore print was not obtained). A dark cream to pale ochre spore print may therefore still be possible and could perhaps match the 'stramineous' color noted by Murrill for *R. obscuriformis*. It can therefore not be excluded for the moment that Murrill's species is a good member of sect. *Xerampelinae*.

The use of the synoptic key by Kibby & Fatto (1990) is quite frustrating in the case of *R. obscuriformis* since the interpretation of many features (in particular cap color, spore print color, peeling and bruising), does not allow for unambiguous coding and therefore does not lead to any reliable identification.

Russula roseiisabellina Murrill, Lloydia 6(3): 216. 1943. figs 7–13

Original description and typification —Pileus convex to depressed, 4.5–6 cm broad; surface glabrous, slightly viscid, the cuticle not readily separable, rosy-isabelline, margin entire, somewhat striate; context rather thick, white, unchanging, sweet, odorless; lamellae adnate, narrow, close, entire, mostly equal, scarcely any forked, white to pallid, becoming brownish where bruised; spores white in mass, globose or subglobose, distinctly echinulate, 6–7 µm; stipe tapering downward, smooth, glabrous, white, unchanging on drying but brownish where bruised, 4–6 × 1–1.5 cm.

Type collected under an evergreen oak on the west shore of the Prairie, near Gainesville, Fla., June 19, 1938 (F 16365). Also collected under oaks at the Tung-oil

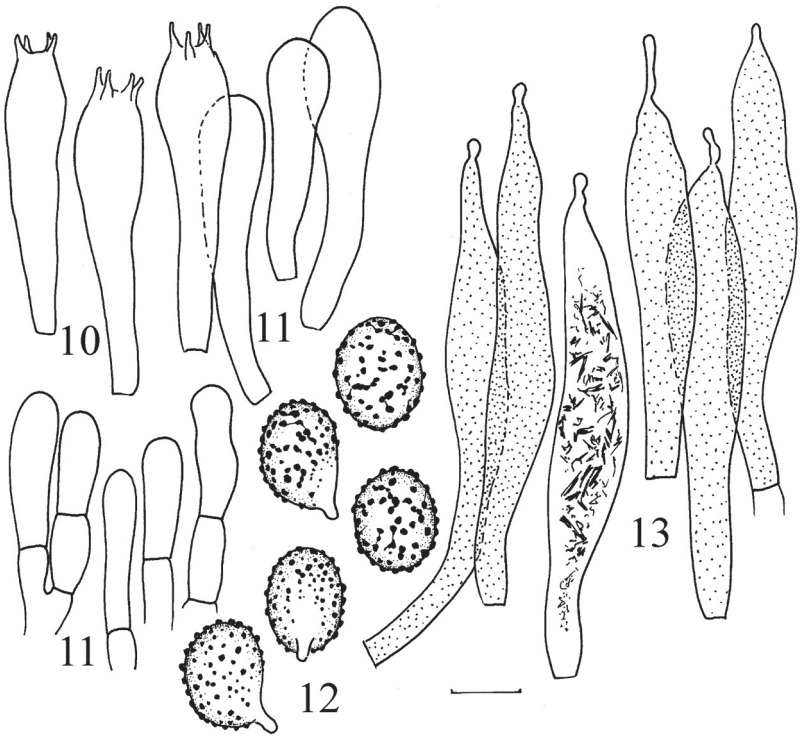


Figs. 7–9. *Russula roseiisabellina* (holotype). 7. Pileocystidia, with contents indicated in one element as seen in Congo red. 8. Hyphal terminations in cap center. 9. Hyphal terminations near cap margin. Scale bar = 10 μm.

Mill, west of Gainesville, June 15, 1938, by Margaret Johnson (F 17209), and in the same locality, June 22, 1938, by West and Murrill (F 17210). This species seems to prefer soil with some lime in it. It is rare, and is found alone or, at most, two near together. [Holotype at FLAS sub nr. F16365].

Microscopic features — Spores ellipsoid, (6.1–)6.7–**7.1**–7.6(–8) × (4.7–)4.8–**5.1**–5.4(–5.9) µm, Q = (1.18–)1.29–**1.39**–1.49(–1.54); ornamentation dens (with 7–10(–13) warts in a 3 µm circle), composed of low and obtuse, amyloid warts measuring 0.2–0.3 µm high, mostly isolated, with some very rare connectives or twinned warts (0–2 fusions in the circle) on some spores; suprahilar plage inamyloid. Basidia (35–)40–**42.6**–45(–46) × (9–)9.5–**10.3**–11(–11.5) µm, 4-spored, clavate. Subhymenium pseudoparenchymatic, small-celled. Lamellar trama mainly composed of large spherocytes. Hymenial cystidia dispersed, often less than 600/mm^2 but more numerous on gill edge, measuring (35.5–)47.5–**56.4**–65.5(–70) × (7.5–)8.5–**10.2**–12(–14) µm, fusiform-pedicellate, mucronate-appendiculate, thin-walled, with SV-negative, crystalline-granular contents, that are strongly refractive in KOH. Marginal cells undifferentiated. Pileipellis near the surface slightly metachromatic in cresyl blue, not sharply delimited from the underlying context, thin, vaguely divided in a poorly gelatinized subpellis of intermingled hyphae, and a suprapellis of more branched and intricate endings with many pileocystidia near the surface only. Hyphal endings thin-walled, often branched at the first or second subapical cell, overall slightly narrower and with shorter terminal cells in cap center compared to cap margin; terminal cells near margin measuring (12–)15.5–**21.6**–28(–42.5) × 4–**4.8**–5.5(–6) µm, subcylindrical or mostly somewhat narrowing upward, in cap center (12.5–)15.5–**19.8**–24(–29) × (3–)3.5–**3.8**–4(–5) µm, less narrowing but on the contrary more often slightly inflated at the tip and subcapitulate; subapical cells mostly equal in width, often branched. Pileocystidia present near surface only, numerous, unicelled, (23.5–)31–**42.1**–53(–68) × (3.5–)4.5–**5.2**–6(–6.5) µm with the largest one near the cap margin, narrow, fusiform to typically subulate with mucronate tips, thin-walled; contents granular-crystalline, often concentrated in upper half or middle portion of the cystidium, insensitive to sulfovanillin. Clamp connections absent in all parts.

Commentary — Because of the mention of the white spore print, the mild taste, and a weakly striate cap margin in the original description, this species keys out to *R.* subgen. *Heterophyllidia* Romagn. when using most keys for European russulas (Bon 1988, 2002a,b; Romagnesi 1985). Using the synoptic key of Kibby & Fatto, the easy and straightforward coding of features (FHKNPTV) leads directly to *R. decora* Shaffer. The latter species is a member of the *R. nigricans* group (*R.* subgen. *Compactae* (Fr.) Bon) and therefore absolutely not a close match for Murrill's type.



Figs. 10–13. *Russula roseiisabellina* (holotype). 10. Basidia. 11. Basidiola. 12. Spores in Melzer's reagent. 13. Hymenial cystidia with contents indicated in one element as seen in Congo red.
Scale bar = 5 μm for spores, 10 μm for the other elements.

Our own observations on features of spores and particularly of pileipellis place *R. roseiisabellina* without any doubt in *R.* subgen. *Ingratula* Romagn. (perhaps close to *R. ventricosipes* Peck): the non-amyloid suprahilar spot, the slender, narrow, mucronate pileocystidia, the weak metachromatic reaction of the suprapellis elements in cresyl blue, and finally the spore ornamentation consisting of low, mostly isolated and obtuse warts are all well known features for other taxa in this group. The metachromatic reaction in cresyl blue and the type of spore ornamentation may also remind of *R.* subsect. *Cyanoxanthinae* Singer (*R.* subgen. *Heterophyllidia*) but the latter species group differs by a different and very characteristic type of pileocystidia and in having strongly incrusted, metachromatic hyphal ends, which are quite different from the ones observed here.

R. roseiisabellina therefore appears to be a good and perhaps rare species that does not correspond to any of the already described members of subgen. *Ingratula* in North America. Whether it is very characteristic in the field is unlikely: our examination of the second specimen identified and mentioned as such in the protologue by Murrill reveals a clearly different species of the *R. foetens*-group with very different spores.

Hesler (1960), who has published a "type-study" with some notes on the spores and pileipellis of *R. roseiisabellina,* describes a quite different type of pileipellis, reminiscent of *R. virescens* (Schaeff.) Fr., and concludes that Murrill's species is identical to *R. rimosa* Murrill, reducing the latter into synonymy. However, as the senior author has had access to Hesler's personal files and duplicate collections at TENN, it is evident that the disagreement between Hesler's and our own observations can be easily explained by the fact that Hesler never examined the type, but relied for his publication on a different, but misidentified specimen sent to him by Murrill.

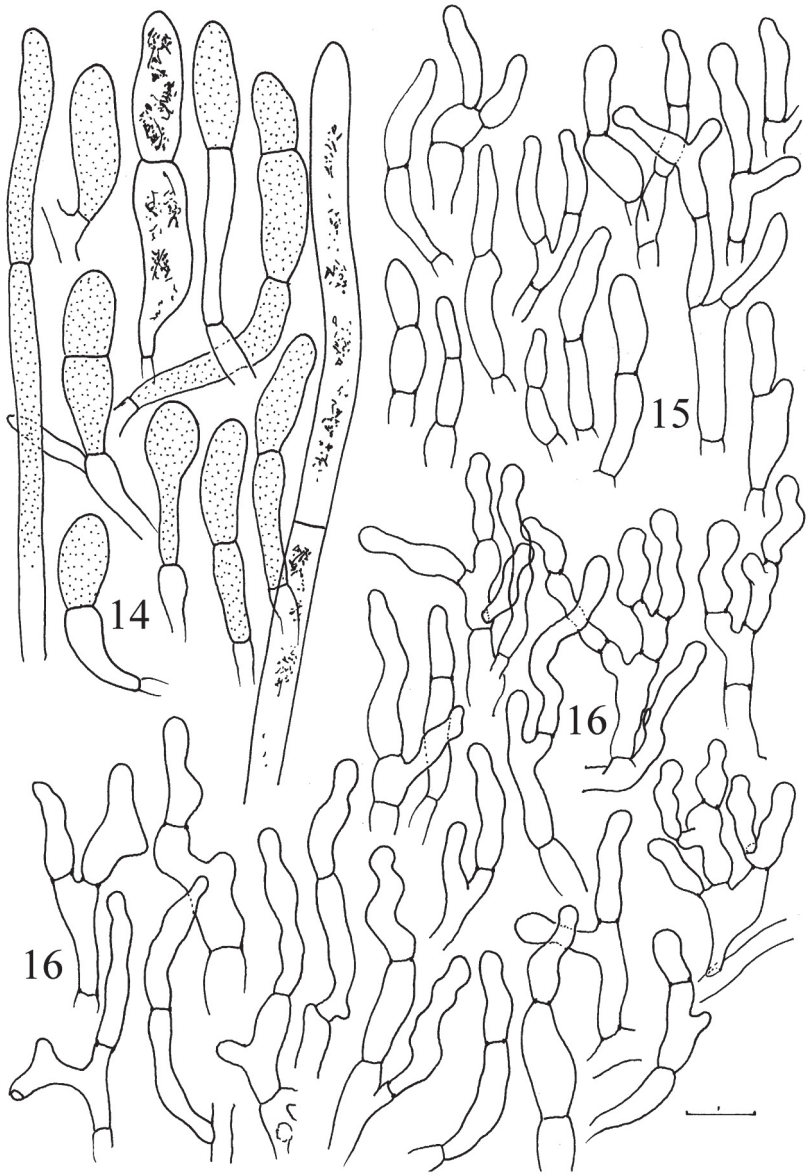
The essential features of *R. roseiisabellina* reside with the pinkish flush on the cap, the pale spore print (described as white, but given the imprecision at that period, a pale cream spore print can not be excluded), mild taste, browning context at least for the gills, and absence of a distinct smell. The importance of its association with *Quercus* on limy soil remains to be verified, but may help in separating it from small individuals of *R. ventricosipes,* a typical pine associate on sandy soil possessing equally a pinkish flush on the cap.

Russula sericella Murrill, Lloydia 7(4): 313. 1945. Figs 14–21

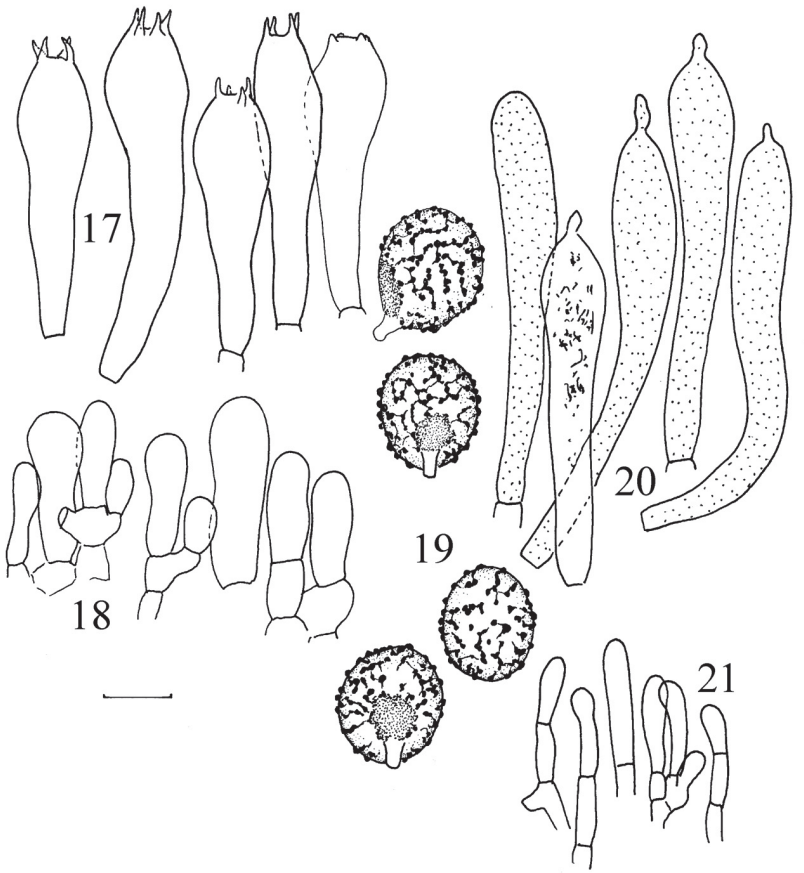
Original description and typification — Pileus convex to depressed, 5–7 cm broad; surface dry, smooth, finely pruinose, vinosous on the disk, paler toward the margin, which is entire and very slightly striate at times; context white, unchanging, odorless, mild; lamellae adnate, many forked at the very base, few inserted, close, narrow, entire, white, gray when dried; spores globose or subglobose, plainly short-tuberculate, white in mass, 6–7 µm; cystidia none; stipe usually equal, stuffed, smooth, glabrous, white, becoming pale-brownish when handled, 5–7 × 1.5–2 cm.

Type collected by W. A. Murrill under a live-oak in Gainesville, Fla., June 1, 1938 (F 12075). Also collected several times under frondose trees in Alachua Co. and once in Clay Co., Fla. Closely related to Kauffman's *R. sericeonitens* but easily distinguished by the change in the color of the stipe. Spores tuberculate; not echinulate as in *R. xerampelina* Fr. [Holotype at FLAS sub nr. F12075].

Microscopic features — Spores subglobose to broadly ellipsoid, (7.3–)7.5–**7.8**–8.1(–8.5) × (6.3–)6.5–**6.8**–7(–7.4) µm, Q = (1.07–)1.11–**1.16**–1.21(–1.35); with a dense subreticulate ornamentation (ca (7–)8–10(–11) elements in a 3 µm circle), composed of broadly conical, amyloid, warts measuring 0.4–0.6 µm high, with numerous line connections ((0–)2–5(–6) connections in a 3 µm circle) or locally fused in short or long ridges (0–4(–5) fusions in the circle); suprahilar plage amyloid. Basidia (37–)39.5–**42.2**–45(–48) ×



Figs. 14–16. *Russula sericella* (holotype). 14. Pileocystidia, with contents indicated in one element as seen in Congo red. 15. Hyphal terminations in cap center. 16. Hyphal terminations near cap margin. Scale bar = 10 μm.



Figs. 17–21. *Russula sericella* (holotype). 17. Basidia. 18. Basidiola. 19. Spores in Melzer's reagent. 20. Hymenial cystidia with contents indicated in one element as seen in Congo red. 21. Marginal cells of gill edge. Scale bar = 5 µm for spores, 10 µm for the other elements.

(10–)11–**11.9**–12.5(–13) µm, 4-spored, clavate. Subhymenium small-celled. Lamellar trama with many very large spherocytes. Hymenial cystidia on sides moderately numerous (1000–1500/mm^2), hardly emergent, originating in the subhymenium, measuring (49–)52.5–**63.3**–74(–89) × (7–)8–**9.3**–10.5(–11) µm, mostly obtuse, occasionally mucronate or appendiculate (up to 4 µm long), thin-walled, with SV-negative contents. Marginal cells on edge of gills very small and slender, hardly differentiated, ca. 7–18 × 2.5–4 µm. Pileipellis orthochromatic in cresyl blue, without incrustations, not sharply delimited from the underlying large spherocytes of the context, thin, vaguely divided in

a rather poorly gelatinized subpellis and a thin, discontinuous and disrupted suprapellis of intricate, small-celled and highly branched hyphal endings; these very thin-walled and also with thin septa; terminal cells very small and short, measuring (8–)12.5–**17**–21.5(–27) × (2.5–)3.5–**4.1**–4.5(–5) µm, mostly very irregular and often nodulose in form, with local contortions and inflations, not attenuated but rounded-obtuse or somewhat inflated near the tips, in the centre of pileus similar, somewhat more regular and with terminal cells of similar size, (8.5–)11.5–**14.7**–18(–20) × (2.5–)3–**3.6**–4.5(–5) µm. Pileocystidia distinct, dispersed, 1–3 celled, of very variable length, mostly situated at the cap surface and with terminal cells 12–**17.5**–23.5 × 5.5–**6.6**–8 µm, ellipsoid, fusiform or rarely clavate, obtuse, but some also longer and cylindrical, ascending from the subpellis, absent in the underlying trama; contents granular to crystalline, refringent, hardly reacting with sulfovanillin. Clamp connections absent in all parts.

Commentary — The only mycologist who published on the type of *R. sericella* was Hesler (1960), unfortunately without any comment on the quite distinctive pileipellis. Singer also examined the type as evidenced by the note he left with the type specimen stating that "*R. sericella* = *R. obscuriformis* Murrill = *R. xerampelina*". We disagree completely with the proposed synonymy, these three taxa being very different as shown here.

Using the synoptic key of Kibby & Fatto (1990) the combination of A?KNPT (with the question mark allowing for variation in our interpretation of the second feature: the "readily" peeling cap) leads either to *R. rosea* Quél. (choosing I = ⅓–¾ peeling) or to *R. sericeonitens* Kauffman (choosing J = ¾ to completely peeling), the latter being indeed the species that is suggested by Murrill himself as being very close to *R. sericella*. Both species are quite different from *R. sericella*.

Use of European *Russula*-keys results in similar placements as for some of the species discussed above and leads to the mild or faintly acrid species around *R. krombholzii* Shaffer in *R.* subgen. *Russula* when using Romagnesi's key (1985) or to a placement in *R.* subgen. *Tenellula* Romagn. with Bon's key (2002).

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